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A unique model of gravity assisted solvent free microwave based extraction of essential oil from mentha leaves ensuring biorefinery of leftover waste biomass for extraction of nutraceuticals: Towards cleaner and greener technology

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ABSTRACT:

The rising discourse over concerns on climate change due to non environmental compatibility of traditional industrial processes have fuelled the development of digitized, high performing, ecofriendly technologies which are in tandem with environment. In the context of extraction of essential oil, drawbacks of classical hydrodistillation such as thermal degradation, high energy consumption, time consuming, low yield have made way for more greener approach. The novelty in the proposed work lies in the development of a rapid, solvent free, gravity assisted microwave based extraction model. The model ensures complete bio-refinery of the residual biomass for extraction of other non-volatile principles with special emphasis to nutraceuticals. The developed working protocol is based on a novel approach of delivering a high power microwave surge concept. An initial high power microwave surge at 60% power level was applied for 2 min, followed by a surge at 40% power level for 2 min and finally followed by sustained microwave firing at 20% power level for 6 min. The above conditions resulted in extraction of 10 mL (dehydrated) of oil from 60 g fresh mentha leaves. The yield of oil was 5 times higher than conventional hydrodistillation method for 6 h. The results of extraction yield were found to be in tandem with the oil quality (volatile principles and biological activity). The biomass obtained after extraction of oil retained the phenolics, flavonoids and other nutraceutical principles thus making it suitable for re-extraction.

1. Introduction

Recent times have witnessed an increasing demand of low cost, effective, fast and green extraction methods by food, cosmetic, pharmaceutical, beverages and perfumery industries which are capable of reducing extraction time, solvent consumption and can significantly lower environmental pollution and energy costs (Chemat et al., 2015). This has resulted primarily because of the growing concerns regarding environment. For the extraction of essential oil, various conventional extraction methods are used by industries such as steam distillation and hydrodistillation (HD) which have some serious disadvantages such as reduced extraction efficiency, loss of volatile principles, thermal degradation of oil and long extraction period resulting in high consumption of energy resources (Boutekedjiret et al., 2003). Other physical methods include expression which is squeezing out the oil by applying mechanical pressure and dry distillation (Bousbia et al., 2009a). All these methods are non-automatized and its accuracy depends largely on the experience and handling technique of the operator.

This work in particular is committed towards encountering the problems associated with traditional methods with a green approach. In this regard an innovative gravity assisted solvent free microwave assisted extraction (SFMAE) technique [also known as microwave hydrodiffusion and gravity (MHG)] for the extraction of essential oil from *Mentha spicata* (spearmint) leaves was developed by using a strategy of puncturing the oil glands with high power

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microwave firing followed by slow and steady gravity assisted draining of oil from the punctured oil glands using sustained low power microwave firing. Noteworthy, to mention that usage of high microwave power alone for longer duration could have burned the biomass and also could have caused degradation of volatile principles as well (Benmoussa et al., 2018). Also low microwave power alone may not allow recovering the totality of essential oil (Binello et al., 2014). The absorption of microwave energy by the oil glands leads to internal heating resulting in rupture which is effectively executed out using high power microwave firing for a very short period of time.

Such strategy was planned taking into account the immense therapeutic and commercial potential of mentha oil so that industrial production of mentha oil could be increased. In case of conventional HD method, the marc after extraction is discarded or at the maximum may be used for compost generation, dried and used as biomass fuel to produce fire for generating steam during HD process or may be used for making of incense stick which is a commonly used commodity for performing worship in India. Availability of plant bioamass for extraction of bioactives is a critical issue as it is linked with conservation of biodiversity and prevention of over exploitation of a particular plant species. In light of the above fact, a deep need for an extraction model is felt which can serve twin purpose which is extraction of volatile oil subsequently followed by extraction of non-volatile bioactives from the same biomass. This shall lead to complete judicious utilization of biomass and also can address biodiversity issues. The key feature of this novel approach was ensuring complete re-use of the left over biomass after oil extraction, for extraction of nutraceuticals. Thus the waste of one (perfumery industries) becomes the precursor for the other (nutraceutical/dietary supplements industries). The above concept is not admissible in case of conventional HD where water is commonly used, because during the extraction process, polar components can leak out into water (extraction solvent). Moreover, continuous boiling of the raw material in water may result in degradation of phyto-constituents. Henceforth, the marc left over after HD cannot yield appreciable quantity of non-volatile bioactive(s) if re-extracted again. The SFMAE model has been attempted here in this case to serve twin purpose, whereby the extraction of volatile oil from mentha leaves can be accomplished within quick time without using any solvent and subsequently the marc can be re-extracted using soxhlet extraction for obtaining other non-volatile bioactives.

Spearmint belonging to lamiaceae family is a perennial herb and cultivated in all parts of the world. It is best known for its flavoring and fragrance property extensively used in food, cosmetics and tobacco industry (Choudhury et al., 2006). In India the fresh leaves are crushed and consumed as "sauce/salad" along with main course diet. It is also reported for its antioxidant, anti-inflammatory, analgesics and antimicrobial property (Kanatt et al., 2007).

According to a report published in leading Indian business newspaper economic times, India contributes 80% of total mentha oil global production apart from China, Brazil and USA. As per the report "Essential Oil – Global Industry Perspective, Comprehensive Analysis and Forecast, 2014–2020," the global demand of essential oil will increase largely in the coming years (ET Markets, 2017). Henceforth, with so much of commerce and trade associate with this commodity, it was thought worthwhile to design a rapid, green and eco-friendly extraction technique for the large scale extraction of mentha oil which can be a boon for the food as well as perfumery industries. Moreover, considering the resolutions passed in the Paris convention for climate change regarding carbon load, only such technologies can sustain which are in tandem with environment. This work is also committed towards meeting the resolutions of United Nations Paris convention for climate change (2015).

2. Materials and methods

2.1. Raw material

Mentha spicata leaves were purchased from local vegetable market at Bilaspur (C.G), India. The collected part (Fresh leaves) was washed with distill water and subjected for extraction after physically draining off water. The moisture content of the sample was found to be 80 ± 0.1%. Commercially available mentha oil in the brand name "Peppermint Oil" manufactured by "S.K. Products, Meerut (U.P), India" with Batch number P085 was purchased from the local market.

2.2. Chemicals

Folin-denis reagent, gallic acid, standard menthol and 2,2-Diphenyl-1-picrylhydrazyl (DPPH) was purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). Quercitin, rutin, ascorbic were purchased from HIMEDIA Co. Ltd. (India). All solvents used in the extraction and chromatography processes were purchased from SD Fine Chemicals Pvt. Ltd (India).

2.3. Apparatus

Microwave extraction was performed with a commercially available microwave extractor (CATA R-invert) manufactured by Catalyst Systems (Pune, India). The extraction system comprised of a microwave extractor, equipped with a magnetron of 2450 MHz with a nominal maximum power of 850 W which can be varied upto 10 different power levels, a time controller, a powerful exhaust system, a beam reflector and condenser attached to a laboratory recirculating chiller [Fig. 1]. An oval glass extraction vessel (2 L capacity) half filled with fresh leaves was kept in an inverted mode inside the microwave extractor. The narrow neck of the extraction vessel connects with a condenser attached below the extraction chamber. The oil oozes out from the ruptured oil glands and flows down by the action of gravity through the condenser and gets collected in the collection vessel attached at the bottom of the condenser. A Camag (Switzerland) HPTLC system with a Linomat 5 injector and TLC scanner 3 was used with WinCATS version 1.4.4 for

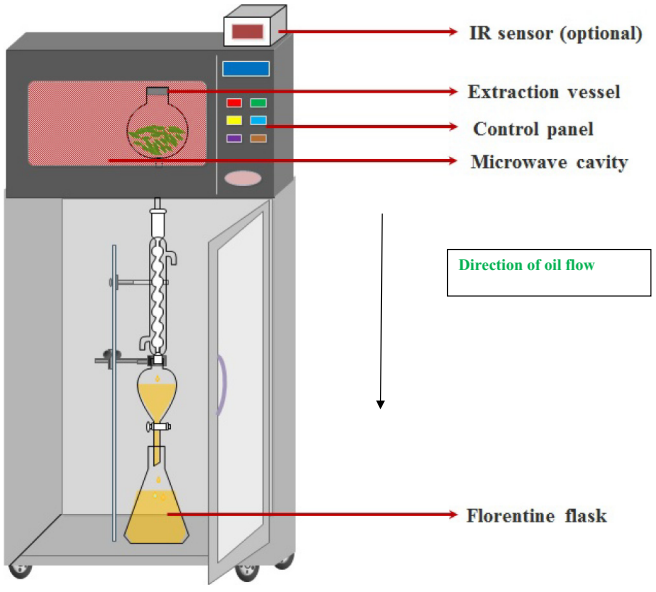

Fig. 1. Schematic diagram representing the operational set-up for SFMAE.

quantification. Analytical Technologies (Baroda, India) Binary Gradient HPLC system (P3000) was used for HPLC analysis. Automated Soxhlet Extraction unit B811 (Buchi, Switzerland) was used for the extraction of mentha leaves. The machine was operated under "Soxhlet warm mode" whereby both the collection beaker and extraction thimble are heated simultaneously in a controlled fashion.

2.4. MAE protocol

60 g of fresh leaves cut into small pieces was accurately weighed and loaded into the extraction vessel without addition of any solvent. An immediate surge of high microwave power (60% = 510 W) was applied for 2 min followed by natural cooling of the system and then subsequent surge of 40% (340 W) microwave power for 2 min was applied. The basic idea was to facilitate quick rupture of oil glands. The system was allowed to cool down naturally which was followed by sustained microwave firing at 20% power level (170 W) for different time intervals carried out at an increment of 2 min as explained in Table 1. Sustained microwave firing at 20% power level (after initial surge of high power microwave) was carried out for 2, 4, 6, 8, 10 and 12 min, every time fresh material was loaded and experiment was progressed after applying the initial surge conditions. Noteworthy, to mention that intermittent cooling period was allowed after every 2 min of microwave firing. The process of sustained microwave firing was carried out till physically no collection of oil was observed. The oil so collected at the end of the extraction was dehydrated by passing through anhydrous sodium sulphate and then stored at 4 °C in sealed centrifuge tubes for further analysis. The density of the oil was calculated using a pycnometer. Extraction yield was calculated as given in equation (1).

$$\text{Extraction Yield } (\%) = \frac{\text{Weight of oil (g)}}{\text{Weight of the raw material (g)}} \times 100\% \quad (1)$$

2.5. Conventional hydrodistillation (HD)

60 g of *Mentha spicata* herb was subjected to HD in a classical clevenger apparatus and extracted with 1L of water for 6 h (until no more essential oil was obtained). A laboratory re-circulating chiller operated at 10 °C was connected to the condenser of clevenger apparatus. The oil so collected at the end of the extraction was dehydrated by passing through anhydrous sodium sulphate and then stored at 4 °C in sealed centrifuge tubes for further analysis.

2.6. Total phenolic content (TPC) and total flavonoid content (TFC)

Detection of total phenolics and flavonoids from essential oil has been vividly explained by Tohidi et al. (Tohidi et. al., 2017) and detection of the same from crude extract has been previously explained by Kala et al. (Kala et al., 2017). Total phenolic content of essential oil and extract was determined by the Folin–Ciocalteu method. TPC was expressed as gallic acid equivalent (GAE) in μg per g of oil for oil and mg per g of dried extract for extract from the calibration curve of gallic acid standard solution. For gallic acid, the calibration curve ($y = 0.036x - 0.012$, $r^2 = 0.995$) was established by preparing standard solution of gallic acid (10, 20, 40, 60 and 80 μg/mL in methanol).

TFC of essential oil was determined by $AlCl_3$ method (Kala et al., 2017) using quercetin as standard and results were expressed as quercitin equivalent in mg per g of oil and mg per g of dried extract for oil and extract respectively from the calibration curve of quercetin standard solution ($y = 0.006x + 0.005$, $r^2 = 0.994$).

2.7. Antioxidant activity

The antioxidant activity was carried out using DPPH method as per the method described by Sharififar et al. (2007) with slight modification. 0.1 mM solution of DPPH and 3 mL sample solution at a fixed concentration (2 mg/mL for oil and 100 μg/mL for extract) was used. After an incubation period of 30 min at room temperature in dark, the absorbance was read against a blank at 517 nm.% scavenging activity was expressed as in equation (2)

$$\text{Scavenging activity } (\%) = \frac{\text{Ablank} - \text{Asample}}{\text{Ablank}} \times 100\% \quad (2)$$

For real time screening of antioxidant compounds the dot-blot test (Mimica-Dukic et al., 2003) using TLC silica plates being stained with purple colour DPPH solution was used. 5 μL of the sample (oil/extract) at a fixed concentration was applied on the TLC plate and developed using the solvent system toluene: ethyl acetate: formic acid: methanol in the ratio of 6: 6: 1.6: 0.4 (v/v). The developed plate was then dipped in developing tank containing 0.4 mM DPPH solution. Yellow spots formed on the TLC plate from bleaching of the purple colour of DPPH reagent, were evaluated as positive antioxidant activity.

2.8. Total protein & carbohydrate estimation

Leaf samples (marc after oil extraction) of 1 g from each treatment (SFMAE and HD) were homogenized in 2 mL of 50 mM Tris-

Table 1
Extraction kinetics and effect of microwave surge strategy on the yield of mentha oil.

Experimental runs	SFMAE	Qty of oil (mL) per 60 gm of fresh weight of mentha leaves	Extraction yield (% w/w)	AUC of menthol (HPTLC)
Initial conditions: SFMAE with high power microwave surge strategy. Extraction conditions with microwave surge: 60% microwave power surge for 2 min + 40% microwave power surge for 2 min.				
1	2 min +20% power	8 ± 0.4	12.1%	659 ± 14.4
2	4 min +20% power	9.5 ± 0.4	14.4%	681.8 ± 15.1
3	6 min +20% power	10 ± 0.3	15.2%	986.1 ± 17.7
4	8 min +20% power	9 ± 0.3	13.7%	685.5 ± 15.4
5	10 min +20%power	8 ± 0.5	12.1%	562.4 ± 17.1
6	12 min +20% power	8 ± 0.5	12.1%	469.7 ± 14.1
SFMAE without any initial microwave surge. Extraction conditions: 20% microwave power, time: 2,4,6,8,10,12 min.				
1	2 min +20% power	0.5 ± 0.1	0.8%	137 ± 9.1
2	4 min +20% power	0.7 ± 0.1	1.12%	208.2 ± 9.2
3	6 min +20% power	1 ± 0.2	1.6%	292.7 ± 10.9
4	8 min +20% power	1 ± 0.2	1.6%	339.5 ± 11.9
5	10 min +20%power	2 ± 0.2	3.2%	372.9 ± 12.1
6	12 min +20% power	2 ± 0.2	3.2%	399.4 ± 12.8

HCl buffer, pH 7.2, containing 0.1 mM ethylenediamine-tetra acetic acid (EDTA) along with 1% w/v polyvinyl-pyrrolidone at 4 °C. Centrifugation of the so obtained homogenate was carried out at 10000 × g for 15 min and the resultant crude supernatant was used for protein estimation (Achary et al., 2012). The concentration of proteins was determined by means of Bradford method (Bradford, 1976). The absorbance was measured at 595 nm spectophotometrically. Bovine Serum Albumin was used for the calibration curve. Carbohydrate estimation was carried out using anthrone reagent method as described by James (1995) using glucose as the standard and absorbance were recorded with a UV spectrophotometer at 620 nm (James, 1995).

2.9. Chemomicroscopy

Transverse sections of the central midrib portion of mentha leaves were taken manually and dipped in Neu's reagent (Khadhraoui et al., 2018) and observed under fluorescent microscope (Axio Cam ERc 5s, Carl Zeiss, Germany, Filter: FITC) for real time *in-situ* detection of phenolic/flavonoids (visible as bright green fluorescent spots in a dark background of leaf section) directly in the leaf tissue. Fresh leaves (control sample) and leaves (marc) left after extraction of oil through SFMAE and HD methods were subjected for chemomicroscopic studies.

2.10. Chromatography analysis

2.10.1. HPTLC analysis

Detection and quantification of menthol present in the essential oil were carried out using high performance thin layer chromatography (HPTLC) as per the method of Alam et al. (2016). The R_f value of the peaks obtained in the sample extracts were compared with that of authentic external standards. Methanolic solution of menthol (standard compound) of known concentrations and essential oil sample were applied onto pre-coated silica gel 60 F_{254} aluminum plates (20 cm × 10 cm), positioned 10 mm from the bottom and 15 mm from the side of the plate, with an 8 mm band width, using a Camag Linomat 5 automated TLC applicator with nitrogen flow providing a delivery speed of 150 nL s^{-1} from the syringe. The mobile phase used was Hexane: Ethyl acetate in the ratio of 8:2 (v/v) for the quantification of menthol. Quantification was carried out at 366 nm and the results were analyzed using WinCATS software.

2.10.2. GC-FID analysis

Mentha oil was analyzed by using gas chromatography (NUCON-5765 GAS CHROMATOGRAPH, Mumbai, India) with a flame ionization detector (FID) and DB-5 capillary column of 30 m × 250 μm × 0.25 μm film thickness (Reddy et al., 2017). Helium (99.99%) was used as a carrier gas at a flow rate of 1.0 mL/min. For the column, the gradient temperature program was maintained at 4 °C/min with a temperature range from 50 °C to 260 °C. In-house analytical standards (for GC peak identification) were provided as complimentary samples by Nishant Aromas, Manufacturer and Exporter of essential oils.

2.10.3. HPLC analysis

Ascorbic acid was quantified by HPLC using mobile phase composition of 0.2% metaposphoric acid in water/methanol (90:10) with detection being carried out at 254 nm (Mandal et al., 2017). Identification of ascorbic acid was done by chromatographic comparisons with authentic standard.

2.11. Field emission scanning electron microscopy (SEM)

SEM images of dried powdered of mentha leaves were obtained for

(a) Control sample: untreated dried mentha leaves
(b) SFMAE: leaves from which oil had been extracted using gravity assisted SFMAE (with surge strategy)
(c) HD: leaves from which oil had been extracted using HD

All the specimens were examined with a JEOL JSM-7610F (Tokyo, Japan) scanning electron microscope under high vacuum condition and at an accelerating voltage of 5.0 kV. Electron gun: Schottky type field emission (T-FE) gun.

2.12. Statistical analysis

All experiments were carried out in triplicate and the means were compared using Student's *t*-test and the Duncan multiple range test (SPSS). P-Values < 0.05 are considered significant. All statistical analyses were performed using the free online statistical software Graph Pad Prism version 7.0.

3. Results and discussion

3.1. Extraction yield of essential oil and effect of microwave surge

When it comes to optimization of microwave based extraction of non volatile bioactives, an extensive optimization involving several factors namely, microwave power, irradiation time, extraction kinetics, effect of solvent, loading ratio, pre-leaching time and many more comes into action and the entire research revolves around the optimization of such critical factors. But for solvent less microwave based extraction assisted by gravity, the optimization basically revolves around two important factors namely, microwave power and irradiation time. In many earlier published reports, (Boukroufa et al., 2015; Bousbia et al., 2009a,b) the microwave power and time has been critically optimized for obtaining the maximum yield of oil and it has been generally seen that operation at high microwave power for longer duration may compromise the oil output and quality and can also burn the matrix (Benmoussa et al., 2018; Jacotet-Navarro et al., 2016). Henceforth, a strategy was thought of for immediate puncturing of oil glands by applying high microwave power surge for shorter duration followed by sustained microwave firing at lower power level. The basic objective was extraction of maximum oil and at the same time safeguard the matrix so that it can be effectively utilized in the second phase for the extraction of other non-volatile principles (with emphasis to nutraceutical components) after the completion of oil extraction.

The yield of dehydrated essential oil obtained by providing initial surge of microwave radiation at high power followed by sustained microwave firing at low power for 2, 4, 6, 8 and 10 min is depicted in Table 1. The density of the oil was found to be 0.91 gm/mL at 25 °C. Results as depicted in Table 1 clearly indicate that highest content of oil (10 mL) and extraction yield of 15.2% w/w was obtained from 6 min (2 min × 3) of gravity assisted SFMAE operated at 20% sustained microwave power upon being effected by 4 min (2 min of 60% microwave power followed by 2 min of 40% microwave power) of high power microwave surge strategy. Upon further continuation of extraction (to the point where no visible collection of oil could be observed) a 20% decrease in extraction yield was observed which could be accounted to the decomposition phenomenon or combustion of essential oil which is unavoidable (Benmoussa et al., 2018; Binello et al., 2014). Degradation due to

pyrolysis of some volatile oil can also be not ruled out (Kokolakis and Golfinopoulos, 2013). The extraction was continued at an increment of 2 min (with fresh leaves being loaded at every 2 min interval) until no visible collection of oil was observed. Based on the above fact, 12 min was determined to be the final end time beyond which the experiment was not continued. The entire extraction can be divided into three major phases post surging phenomenon. The first phase (0–2 min at 20% microwave power, Table 1) post surging represents 80% yield of essential oil which basically occurs because of the immediate rupture of oil glands due to high microwave power surge for shorter duration. The oil is spilled out from the glands on the vegetable surface which then drains into the collection vessel by the action of gravity (Farhat et al., 2010). The second phase of the extraction (4–6 min at 20% microwave power, Table 1) indicates 20% extraction yield which basically represents the internal diffusion of the so liberated oil from inside of the cellular matrix to the outside external surface which is further facilitated by the flow of vapours created from in-situ heating of water content. The last phase of extraction beyond 6 min extraction marks the end of extraction which is signified by no increase in extraction yield, rather degradation of oil is seen which is indicated by reduction in extraction of oil volume. Similar bifurcation to explain different stages of extraction has been reported in the past and the current observation is in agreement with the past reports. (Benmoussa et al., 2018; Boukroufa et al., 2015)

In order to evaluate the effect of high power microwave surge, the same set of experiments as mentioned above was performed but without affecting the initial microwave surge conditions. Results as depicted in Table 1 indicate that there was a significant difference in the extraction yield of mentha oil and almost 5–10 times more yield was obtained when microwave power surge was affected than when compared to gravity assisted SFMAE without power surging. In the case of gravity assisted SFMAE without power surge, after 12 min no visible collection of oil was observed and extraction was not continued beyond this point. The highest amount of oil (2 mL, extraction yield: 3.2% w/w) was collected in 10 min (2 min × 5) of microwave extraction which was 5 times lesser than the optimal extraction yield when high power microwave surge was affected. The low yield when microwave surge is not applied can be accounted to the fact that low power (20% alone) is not sufficient in recovering the totality of essential oil, whereas too high power for excessive longer duration can involve the risk of destroying the vegetable matter (Boukroufa et al., 2015). The above facts clearly indicate that the initial surge of high power microwave compromises the integrity of the oil glands and cellular structures which stores oil. The intense thermal stress that develops due to rapid delivery of high power microwave energy causes the moisture inside the plant cellular structures to evaporate (due to heat generated from microwave absorption) leading to pressure build-up from inside the cells ultimately causing rupture of glands (Farhat et al., 2010). The oil then leaches out from the compromised cellular structures and flows into the collection vessel under gravity. Kala et al. (2017) in their earlier published reports have presented SEM (scanning electron micrographs) evidences to establish the so mentioned cellular rupture theory upon microwave heating.

3.2. TPC and TFC of mentha oil

In order to map down the effect of increased extraction yield with the actual quantitative evaluation of the contents of the oil, TPC and TFC of the so extracted oil was determined. The results are depicted in Fig. 2. The results are in agreement with the previous finding of 6 min being the optimum extraction time in terms of optimal collection of oil volume and extraction yield. Fig. 2 reveals that for extraction of flavonoids, increase in yield of oil also resulted in increased extraction of flavonoids and vice-versa. But for phenolics, increase in yield of oil had no effect on the phenolics content. The rise and fall in the yield of flavonoids in the so extracted oil was very sharp when compared to yield of phenolics during SFMAE (with surge strategy). For the yield of phenolics content, there was no significant difference in TPC values upto 6 min (optimal extraction time) indicating early extraction of phenolics immediately after applying the surge conditions. However, the oil obtained from 6 min of SFMAE with initial microwave surge yielded the highest TPC and TFC and a significant decrease (student's *t*-test,

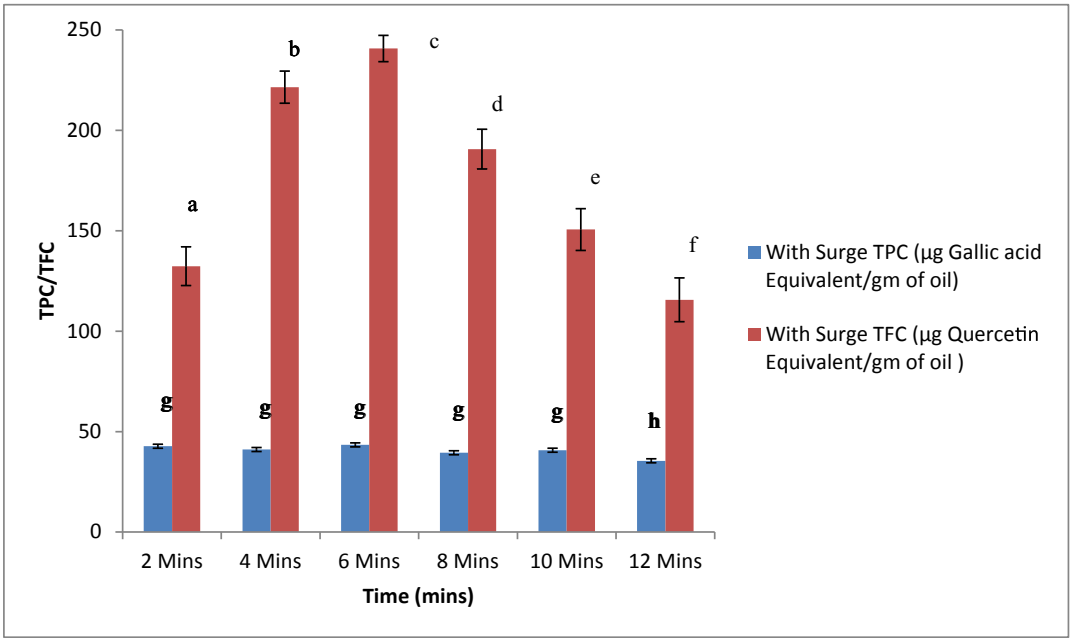

Fig. 2. Effect of high power microwave surge strategy on the yield of TPC and TFC of mentha oil Data marked with different letters are significantly different at $p < 0.05$. Results are expressed as mean ± SD (n = 3).

$p < 0.05$) in TPC to the intensity of 18.3% and TFC content to the intensity of 52% was observed beyond 6 min (optimum extraction time). This fact of drop in TPC and TFC contents synchronizes well with the degradation claim of oil made above to account for the 20% reduction in extraction yield of mentha oil beyond the optimal extraction time of 6 min. The application of microwave surge conditions were kept at the minimum because operating at high microwave power for longer duration may result in degradation of oil components (oxygenated compounds) which eventually becomes evident from the decrease in intensity of the typical smell of the oil and may also result in burning of the matrix (Jacotet-Navarro et al., 2016; Meullemiestre et al., 2014a). The prime objective behind applying microwave surge strategy was to safeguard the matrix so that it can be re-used for the extraction of other non-volatile principles.

Noteworthy, to mention that TPC and TFC could not be estimated spectrophotometrically for the oil collected from microwave extraction where initial high power microwave surge was not affected and oil collected from HD. This could be accounted due to very low or no extraction yield of phenolics and flavonoids. During HD long treatment time and localized over heating can cause potential degradation or hydrolysis resulting in poor quality of essential oil and loss of more volatile compounds. In case of hydrodistillation maximum yield obtained after 6 h of exhaustive extraction was 2 mL.

3.3. Chromatographic analysis (HPTLC & GC-FID)

The content of menthol in the so collected oil was evaluated semi-quantitatively through HPTLC. Results have been expressed as AUC (area under the curve) values (Table 1). A significant difference in AUC of menthol was observed when compared to the oil obtained from gravity assisted SFMAE conditions where high power microwave surge was not applied. Results are indicative for the assumption that increase in extraction of oil volume is directly proportional to its menthol content. Alcohols such as oxygenated monoterpenes due to its hydrophilic nature are well extracted under microwave conditions because of their better interaction with microwaves (Meullemiestre et al., 2014a,b). Past reports provide evidence that microwave extracted oils are richer in alcohols, such as oxygenated monoterpenes or hydroxyl polymethoxy flavones (tangeretin) due to more effective extraction of hydrophilic molecules (Ciriminna et al., 2017; Jacotet-Navarro et al., 2016). Results clearly support the claim for 6 min as the optimal extraction time, (when microwave surge is affected) as the highest menthol content was obtained from oil produced at this particular operating condition and the yield was found to be 62% more when compared to the AUC of menthol for the oil obtained by SFMAE (without surge) at 10 min (optimal extraction time for SFMAE without surge). Moreover, in case of SFMAE (with surge strategy) the yield of menthol (in terms of AUC) dropped by 52.4% when extraction was carried out beyond 6 min till the end point of 12 min. This could be easily accounted to excessive heating of such molecules due to better interaction with microwaves (Jacotet-Navarro et al., 2016). The above facts clearly indicate that extraction of menthol was significantly on the lower side when microwave surge was not affected thus indicating the importance of high power microwave surge in rupturing the oil glands. The above results also indicate the need for optimizing the process as anything beyond the optimum operational conditions could be fatal as severe degradation of oxygenated compounds and other volatile oil components can take place.(Jacotet-Navarro et al., 2016; Meullemiestre et al., 2014a)

Gas chromatography analysis with FID revealed 49 peaks with a cumulative area of 100017.55 for oil extracted using microwave assisted extraction whereas oil obtained from HD yielded 30 peaks with a cumulative area of 31509.52 which was 3 fold lesser than the oil obtained from the proposed method. Using external standards the following important peaks were identified as shown in Table 2. Results clearly indicated that only 6 vital volatile principles were identified in the oil obtained from HD with significant decreased content as evident from the %area analysis. Whereas, the oil obtained from gravity assisted SFMAE showed the presence of 13 vital volatile principles with significant increased content of both oxygenated and non-oxygenated terpenes.

3.4. Evaluation of biomass integrity after extraction of oil to assess its re-usability factor

The integrity of the biomass after extraction of oil either from SFMAE or HD was evaluated by estimating phenolics/flavonoids content, nutraceutical contents and biological potency. After gravity assisted SFMAE (with surge strategy) of volatile oil, the left over marc was vacuum dried and subjected (1 gm) to automated Soxhlet extraction for 60 min using methanol as the extraction solvent (Chouhan et al., 2019). Dual heating of both collection vessel and extraction thimble resulted in quick extraction of non-volatile bioactive(s) from the already compromised cellular structures of the marc. The extract so obtained was dried and evaluated for the determination of phenolics/flavonoids to establish the re-usability of the biomass for extraction of non-volatile bioactives. Marc

Table 2
List of volatile principles identified using GC-FID.

Compounds identified	Retention Time (min)	Area (%)	
		HD (%)	MHDG (%)
α-pinene	5.55	0.466	2.161
Sabinene	6.44	–	0.204
β-Pinene	6.55	–	0.896
Myrecene	6.81	–	0.192
p-Cymene	7.54	–	0.392
Limonene	7.87	2.075	2.871
g-Terpinene	7.96	–	1.184
Menthone	11.52	0.452	0.944
Isomenthone	12.11	–	0.258
Dihydrocarvone	12.65	–	0.248
Menthol	12.83	0.447	2.594
L-Carvone	14.52	8.974	11.542
Caryophyllene	17.79	0.956	1.457
Total identified monoterpene hydrocarbons		2.54	7.9
Total identified oxygenated terpenes		10.829	17.043

obtained after HD was also treated in the same manner as described above. Untreated mentha leaves (fresh leaves collected and dried) were also subjected to automated Soxhlet extraction as described above and was considered as control. The results so obtained were compared between extract obtained from fresh dried untreated mentha leaves (control), extract obtained from marc from which oil has been extracted by gravity assisted SFMAE and extract obtained from marc from which oil has been extracted through HD. Results are indicated in Table 4. No significant change in yield of phenolics was observed in the extract obtained from microwave treated leaves than when compared to control, but for the yield of flavonoids a drop by 4.5% was observed which is not alarming. Whereas, upon comparison with control, the scenario was completely different for the extract obtained from leaves which were subjected to HD. A sharp significant fall to the extent of 58% and 45% was recorded in the yield of phenolics and flavonoids respectively when compared to control which definitely indicates a potential drop in extraction of bioactives (Table 4). This drop in the yield of bioactives for the leaf extract which was earlier subjected to HD could be accounted to the loss of phenolics/flavonoids principles being washed off or degraded from the plant matrix during long hours of boiling with water involved in the process of HD. It can be well anticipated that if phenolics/flavonoids principles are thermally safe inside the plant matrix during the process of SFMAE and subsequently later on can be extracted from the same biomass using any conventional extraction technique, then the same assumption could be also made applicable for other bioactive principles. On the other hand, the same is not admissible for the biomass subjected to traditional HD as prolonged heating in water and also loss of certain polar components into water during the process of HD can severely jeopardize the existence of other polar non-volatile principles in the marc and re-use of the biomass after HD for extraction of other non-volatile polar bioactives may not be economically judicious.

Biodiversity is a major issue in drug discovery from medicinal plants. Industries whose primary raw material is medicinal plants need to ensure judicious use of plant material to prevent over exploitation of any species to the extent that it may bring a particular species to the threshold of being endangered. Traditional HD method is most commonly used technique for extraction of oil by industries. Such method makes use of large amount of energy resources because of long hours of heating involved and also produces a very low yield. The most important part which goes unnoticed is that the biomass after extraction of oil is simply discarded. The proposed SFMAE of mentha oil using the high power microwave surge strategy resulted in yield of 10 mL mentha oil/60 gm fresh weight (extraction yield: 15.2% w/w) within 10 min of SFMAE. Out of the total 10 min extraction time, 4 min was devoted for high power microwave surge (2 min for 60% microwave power surge and subsequent 2 min for 40% microwave power surge) and the remaining 6 min was devoted for 20% low power sustained microwave irradiation.

3.5. Mapping of antioxidant activity

The antioxidant activity was evaluated as a check for biological activity. Since the extraction method involves application of microwaves (electromagnetic waves), there could be doubts that the increase in yield might be compromised with reduced biological potency. It can be anticipated that if the antioxidant potency can be retained even after exposure to microwaves, other biological properties can also be then retained.

The antioxidant activity of mentha oil (obtained through SFMAE and traditional HD) in terms of % radical scavenging activity was evaluated using DPPH model at a fixed concentration of 2 mg/mL and the results were compared with commercially available mentha oil which was considered as the standard control. 65% scavenging activity was obtained at the said concentration for the standard control, whereas 79% scavenging activity was observed for the oil extracted through SFMAE technique resulting in a rise of 21.5% antioxidant activity at the same concentration level (Table 3). Noteworthy, to mention that no antioxidant activity was observed for the oil collected from HD method. The above results clearly indicate that proposed SFMAE method (with surge strategy) not only produces increased yield but also preserves the biological integrity of the product thus abolishing any fear that application of electromagnetic waves could compromise the medicinal value of the finished product. The antioxidant activity could be attributed to the presence of menthol, menthone, isomenthone, pulegone and cineole as earlier reported by Mimica-Dukic et al. (2003). The oxygenated compounds are of more value in the essential oils with its strong characteristics odour and their yield is directly proportional to the antioxidant activity of the oil (Meullemiestre et al., 2014a). Past results states that antioxidant activities for oil were higher from microwave extracted oil when compared to traditional HD method and the current observation are in agreement with past records. For real time detection and mapping of antioxidant compounds present in the oil, the HPTLC pate images obtained from TLC dot-blot method are shown in Fig. 3 which clearly shows yellow spots deemed to be considered as the actual antioxidant zones in both the standard control and oil obtained from SFMAE. However, no such observation was recorded for the oil obtained from HD method. TLC dot-blot results synchronize well with the % radical scavenging activity results.

The antioxidant activity of the methanolic extract obtained from marc (left over leaves after extraction of oil) was also determined using DPPH model at a fixed concentration of 100 μg/mL and compared with methanolic extract of the same concentration obtained from untreated leaves (control samples). No significant difference in % scavenging activity was observed for the extract obtained from marc from which oil has been extracted using SFMAE technique when compared with control sample at the same concentration (Fig. 3). However, a significant drastic fall to the magnitude of 4.5 times in % scavenging activity was observed for the extract obtained from marc from which oil has been extracted

Table 3
Comparison of extraction performance of gravity assisted SFMAE (with and without surge) with HD.

Quality parameters	SFMAE with surge	SFMAE without surge	HD
Optimum extraction time	6 min	10 min	6 h
Optimum yield of oil (dehydrated)	10 mL±0.3	2 mL±0.2	2 mL±0.6
Extraction yield (%w/w)	15.2 ± 0.8	3.2 ± 0.9	3.2 ± 1.3
% radical scavenging activity at 2 mg/mL (control = 65%)	79% ± 0.89	Not detected	Not detected
TPC (μg GAE/gm of oil)	43.37 ± 1.4	Not detected	Not detected
TFC (μg QE/gm of oil)	240.75 ± 6.5	Not detected	Not detected
Density (gm/mL at 25 °C)	0.91 ± 0.05	0.96 ± 0.06	1.03 ± 0.08
Colour of oil	Pale yellow	Very Pale yellow	colourless
Antioxidant zones in TLC dot blot method	Detected prominently	Not detected	Not detected

Table 4
Comparison of biomass integrity after extraction of oil to ascertain its re-usability.

Quality parameters	Control	Extract produced from biomass obtained after SFMAE	Extract produced from biomass obtained after HD
TPC (mg GAE/gm of dried extract)	7.2 ± 0.9^{a}	6.9 ± 0.8^{a}	2.9 ± 0.94^{b}
TFC (mg QE/gm of dried extract)	30.5 ± 1.2^{a}	29.1 ± 1.4^{b}	16.8 ± 1.8^{c}
% radical scavenging activity at 100 μg/mL	$89.2\% \pm 1.7^{a}$	$87.52\% \pm 1.8^{a}$	$19.6\% \pm 2^{b}$
Protein content (mg/gm of fresh weight)	38.27 ± 1.4^{a}	32.42 ± 1.5^{b}	23.87 ± 1.8^{c}
Ascorbic acid content (mg/gm of dried sample)	8 ± 0.2^{a}	7.5 ± 0.3^{a}	2.9 ± 0.5^{b}
Carbohydrate content (% w/w)	2.42 ± 0.13^{a}	1.99 ± 0.18^{b}	0.82 ± 0.21^{c}
Antioxidant zones in TLC dot blot method	Detected prominently	Detected prominently	Not detected

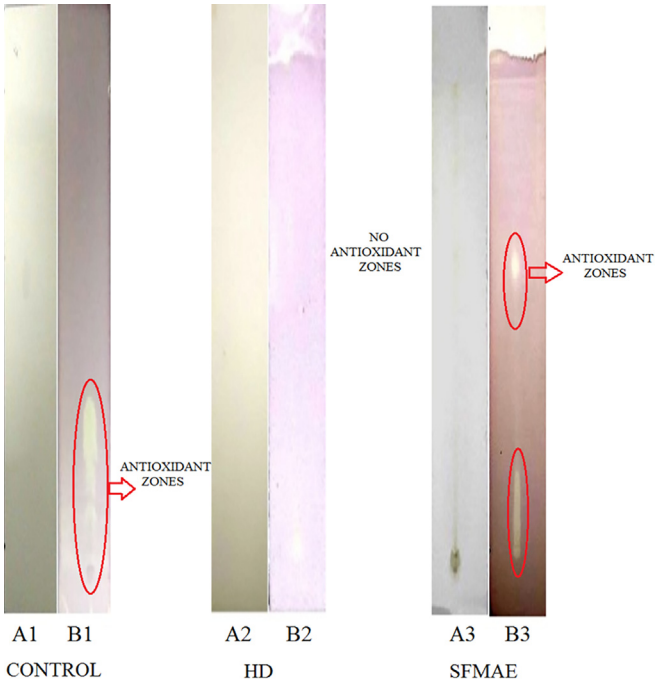

Fig. 3. Plate image of TLC dot-blot test for mapping of antioxidant zones (mentha oil). Control: mentha oil purchased commercially. HD: mentha oil obtained from 6 h of HD. SFMAE: mentha oil obtained through SFMAE (with surge strategy). A1: control plate before dipping; B1: control plate after dipping in 0.4 mM DPPH solution. A2: oil (HD) plate before dipping; B2: oil (HD) plate after dipping in 0.4 mM DPPH solution. A3: oil (SFMAE) plate before dipping; B3: oil (SFMAE) plate after dipping in 0.4 mM DPPH solution.

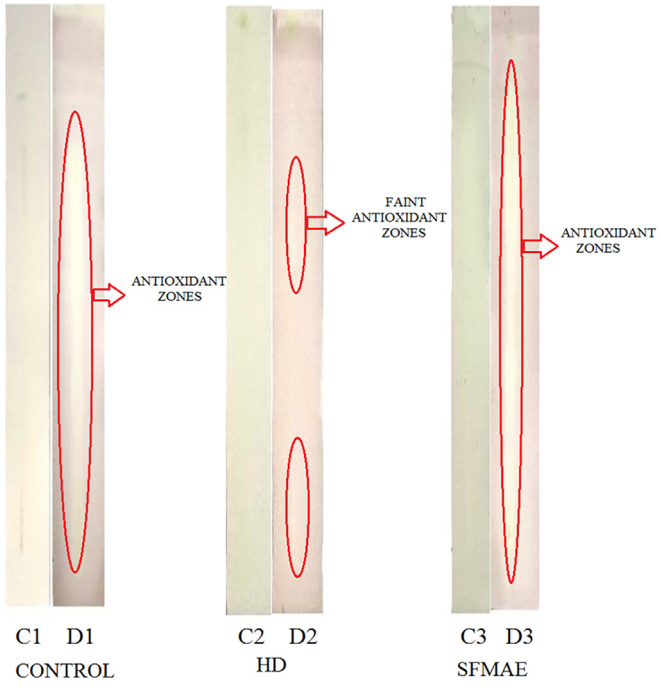

Fig. 4. Plate image of TLC dot-blot test for mapping of antioxidant zones (mentha extract). Control: extract obtained from fresh untreated dried leaves of mentha. HD: extract obtained from re-extraction of the biomass which was subjected to oil extraction by HD. SFMAE: extract obtained from re-extraction of the biomass which was subjected to oil extraction by SFMAE. C1: control plate before dipping; D1: control plate after dipping in 0.4 mM DPPH solution. C2: plate before dipping; D2: oil (HD) plate after dipping in 0.4 mM DPPH solution. C3:plate before dipping; D3: plate after dipping in 0.4 mM DPPH solution.

by HD method when compared to control sample. This fact clearly establishes the claim that even after SFMAE of oil from mentha leaves, the biomass remains safe and productive enough to yield bioactive polar non-volatile principles if reused and re-extracted with suitable solvent. Whereas, on the other hand results clearly indicate that the same is not admissible for the biomass from which oil has been extracted using HD method. During HD method long heating hours takes a severe toll on the non-volatile polar bioactive principles and also the probability of being leached out by water used during HD cannot be ruled out. In a recently published article (Chouhan et al., 2019) the authors have shown significant evidences of phenolic degradation due to long heating hours involved in Soxhlet extraction. For real time detection and mapping of antioxidant compounds, TLC dot-blot method for the extracts was also carried out (Fig. 4). The yellow spots obtained in the TLC dot blot method was quantified using a free online software "Just TLC" (version 4.0.1) whereby the yellow area was traced by the software manually and converted to AUC. There was no significant difference in the AUC of extract obtained from marc from which oil has been extracted using SFMAE technique when compared with control but a fall to the magnitude of 4.3 times was recorded for extract obtained from marc from which oil has been extracted by HD when compared with control. The results are in complete harmony with the reports of % radical scavenging activity. The above fact clearly establishes the claim that the biomass from which oil has been extracted using gravity assisted SFMAE still can be reused for extraction of non-volatile bioactives with yield comparable to that of control.

3.6. Nutritional content analysis

The results of total protein content (Table 4) also indicates that apart from the biological potential and phenolics/flavonoids content, the integrity of the additional nutritional components are also preserved in the biomass from which oil has been extracted out

using SFMAE. Whereas, on the other hand, this fact again is not admissible for the biomass which were subjected to HD (for extraction of oil) as total protein content recorded a fall of 37.5% when compared to control sample. The same observations were recorded for ascorbic acid content also. No significant difference in ascorbic acid content was found between control sample and extract obtained from leaves which were subjected to SFMAE for oil extraction indicating conclusive evidence on retention of nutritional values during extraction of oil using SFMAE technique (Table 4). Noteworthy, to mention that a drop in ascorbic acid content to the magnitude of 2.1 times was recorded for the extract obtained from leaves which were subjected to HD for oil extraction when compared to the control (extract from untreated leaves). Similar observations were also recorded for carbohydrate content (Table 4).

The most interesting part of this proposed SFMAE method is the re-usability factor of the biomass. During extraction of oil the other non-volatile principles are safely retained in the biomass with their biological potency intact which can be later on extracted from the same biomass using suitable solvent extraction method. This fact could be of potential interest for the perfumery and food industries where waste (biomass discarded after extraction of oil) from the perfumery industries could be the starting raw material for the nutraceutical industries for the extraction of nutraceutical principles.

3.7. Chemo-microscopy analysis

Chemo-microscopy was done for real time detection of phenolic/flavonoid principles in the leaf tissue to provide back end support for the TPC/TFC data. When immersed into 1% w/v methanolic solution of Neu's reagent, plant pigments such as chlorophyll can be removed allowing a better localization and visualization of phenolic and flavonoid compounds. The fluorescence intensity of the leaf tissue is directly proportional to its phenolic/flavonoid contents (Khadhraoui et al., 2018). Fig. 5 clearly indicates that comparable fluorescence intensity was observed for control sample and leaf sections from which oil has been extracted using SFMAE. The bright fluorescent spots in the parenchyma and collenchyma region indicate actual localization of phenolics and flavonoids. This gives a real time evidence that phenolics/flavonoids are safely retained in the biomass after extraction of oil through SFMAE and thus again can be re-used for extraction of nutraceutical principles by the food processing industries. The above evidence gives a clear indication that gravity assisted SFMAE is a most efficient technology to save the structure of phenolic groups and to avoid their contributions in side reactions leading to either degradation or formation of undesirable products (Meullemiestre et al., 2014b). Drastic reduction in fluorescence intensity (practically no fluorescent spots were visualized in the parenchyma or collenchyma region) was observed in the transverse section of leaf sample from which oil has been extracted through HD. This reduction in intensity provides true evidence for the earlier claim that phenolics can leach out into water during HD or may also get degraded during intensive boiling for hours involved in the process of HD.

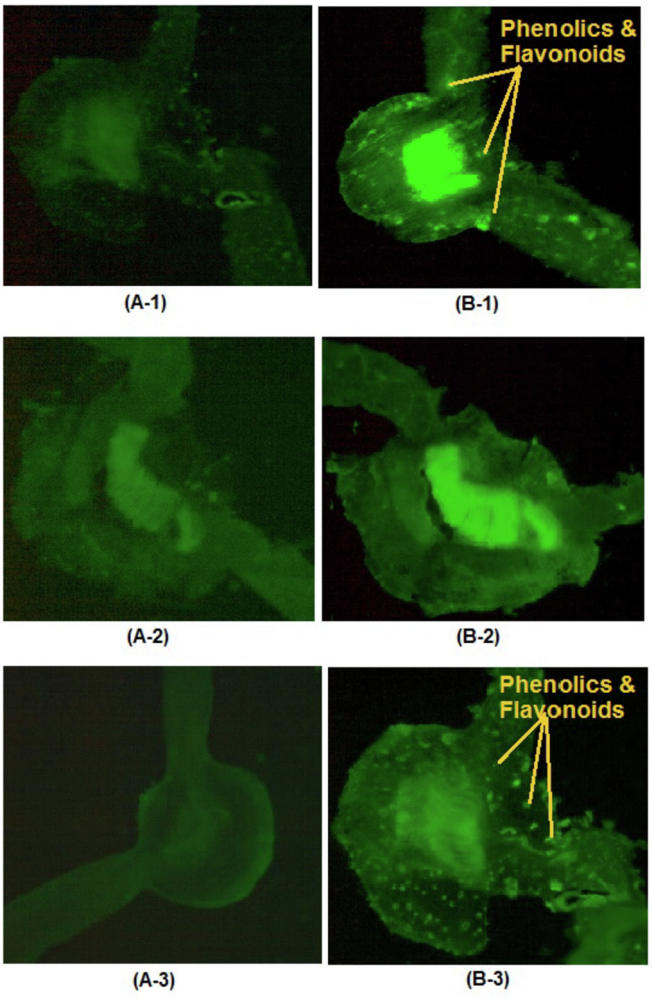

Fig. 5. Chemo-microscopy images under fluorescent light. A-1: untreated control, B-1: Neu's reagent treated. A-2: untreated leaf section (biomass which was subjected to HD for oil extraction). B-2: Neu's reagent treated leaf section (biomass which was subjected to HD for oil extraction). A-3: untreated leaf section (biomass which was subjected to SFMAE for oil extraction). B-3: Neu's reagent treated leaf section (biomass which was subjected to SFMAE for oil extraction).

3.8. HPTLC analysis

3.8.1. Fingerprint comparison

Chromatographic finger print for the extracts at a fixed concentration of 10 mg/mL was obtained through HPTLC by using the mobile phase toluene: ethyl acetate: formic acid: methanol in the ratio of 6: 6: 1.6: 0.4 (v/v) which gives a good resolution for phenolics and flavonoids (Kala et al., 2017). Fig. 6a shows the comparison of HPTLC fingerprint of control sample (extract obtained from fresh untreated leaves) and extract obtained from the leaves from which oil has been already extracted using SFMAE. The pattern of bands and its intensity in the both the extracts appeared identical giving an apparent assumption that the phenolics and flavonoids or other phyto-components are very well retained back in the biomass during the process of oil extraction using microwave surge concept. Similarity in HPTLC band pattern also indicates that no harmfull degradation products were formed due to microwave exposure during oil extraction. If the cumulative area under the curve (AUC) of control sample is to be considered as 100%, the cumulative AUC of the extract amounted to 96% relative to control. Whereas, on the other hand the fingerprint pattern for the extract obtained from leaves from which oil has been extracted using HD method appeared too faint when compared to control sample and could amount to only 40% cumulative AUC relative to control (Fig. 6b). In other words it can be said that 96% retention of non-volatile bio-actives took place in the biomass which was treated with microwaves for oil extraction and thus can be re-extracted again for the extraction of retained bio-actives. Whereas, the retention level of non-volatile bioactives was 40% (depletion level 60%) for the biomass which was subjected to HD for oil extraction

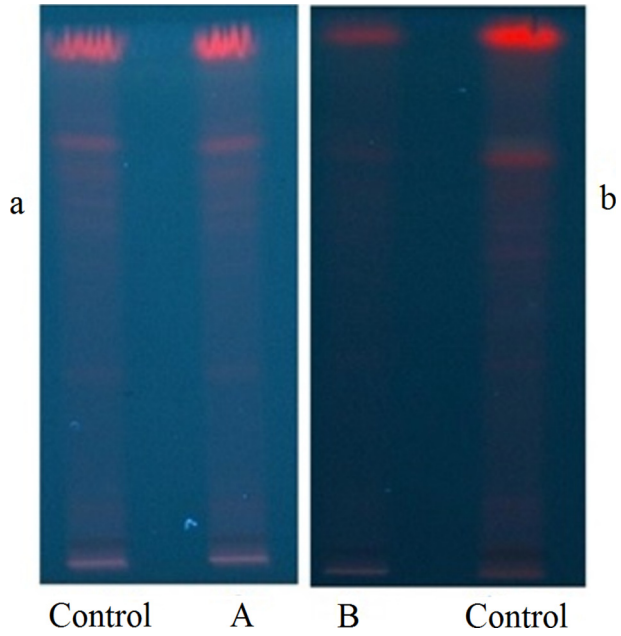

Fig. 6. HPTLC fingerprint comparison. Control: extract obtained from dried untreated leaves. A: extract obtained from re-extraction of biomass from which oil has been extracted using SFMAE. B: extract obtained from re-extraction of biomass from which oil has been extracted using HD method.

indicating a severe depletion of vital bioactives during the process of HD thus making the biomass unfit for re-use.

3.8.2. Semiquantitative estimation of loss of vital non-volatile phenolic/flavonoid principles

The depletion of gallic acid, quercitin and rutin (major bioactives detected in mentha leaf extract) in the methanolic extract obtained by re-extraction of biomass from which oil has been extracted earlier either by SFMAE or HD method was estimated in terms of calculated AUC. If the cumulative AUC of gallic acid, quercitin and rutin is to be considered as 100% for control sample (extract from untreated leaves) then extract obtained from reuse of biomass which was subjected to SFMAE for extraction of oil amounted for 93.5% relative AUC indicating almost complete retention of the above mentioned principles in the biomass after completion of SFMAE, thus making the biomass eligible for re-extraction of vital biocative principles. Whereas, the extract which was obtained from the leaves subjected to HD for extraction of oil could amount for only 55.5% cumulative AUC of gallic acid, quercitin and rutin relative to control, thus indicating a depletion of 45% during the oil extraction by HD method. Henceforth, in the 60% overall depletion of bioactive principles (with emphasis to phenolics and flavonoids) that took place in the biomass subjected to HD for oil extraction, 45% alone could be accounted for the depletion of gallic acid, quercitin and rutin.

3.9. Microstructural changes during the extraction of essential oil

The physical structural changes occurring in the oil glands of mentha leaves upon extraction with microwave and HD are shown in Fig. 7. Images taken at two magnification level are conclusive in evidence and clearly demonstrate total rupture of oil glands during SFMAE which assisted easy drainage of oil from the ruptured glands by the action of gravity (Fig. 7-C & 7-CC). Micrographs also revealed that during HD the oil glands were squeezed, wrinkled and reduced in size (Fig. 7-B & 7-BB) but no conclusive evidence of rupture was seen when compared to the intensity of massive bursting of oil glands for microwave treated sample. This massive bursting can be accounted due to the pressure build up in the oil glands due to the internal heating of “in-situ” water. The “in-situ” water upon absorbing microwave heats up tremendously and the vapours formed develops a pressure from inside and when it exceeds the capacity of expansion for the oil glands, it results in massive bursting of the oil glands which is also assisted by the thermal stress that develops due to volumetric heating of the internal organic molecules present in the glands and vascular systems (Jacotet-Navarro et al., 2016; Benmoussa et al., 2018). Whereas, in case of HD such phenomenon was not observed as it basically operates on the principle of heat transfer regulated by conduction and convection theories and hence rupture of oil glands did not take place. Similar disruption of oil glands due to intense microwave heating has been depicted in the past by several researchers (Farhat et al., 2010; Ma et al., 2012). Moreover, during SFMAE both heat and mass transfer (transfer of oil) takes place from inside the oil gland to the outside matrix surface, whereas during HD both the above mentioned phenomenon takes place in opposite direction. Since in SFMAE both heat and mass transfer are synergistic to each other, the acceleration is magnified enormously. During HD the two phenomenon antagonizes each other and thus slows down the extraction process.

3.10. Comparison with traditional methods

A comparative chart of the proposed SFMAE with conventional HD method is shown in Table 3 and 4 The effect of such extraction technique on the biomass with special emphasis to the integrity of the non-volatile principles has been clearly demonstrated. The table is self explanatory and provides enough evidence on the fact that the biomass left after extraction of volatile oil using SFMAE can be effectively re-used for the extraction of other non-volatile principles and their biological potency is also protected. The appearance of the marc left over after SFMAE was comparable to the control sample whereas the marc obtained after HD appeared wrinkled, blackish and was too brittle to handle. There was no greenness left in the leaves as well. The effectiveness of the proposed SFMAE method with surge strategy is very well depicted in the comparative analysis in Tables 3 and 4

3.11. Environmental impact

Since the operation is fully solvent free so there involves no basic cost for extraction solvent. The only cost factor involved is power consumption. The scalability factor shall be comparatively easier to handle as there involves lesser parameters to be optimized since solvent is not being used in the process. In-fact, industrial feasibility has been successfully reported by authors in the past (Ciriminna et al., 2017). The environmental impact in terms of CO_2 liberated in the atmosphere for each mL of oil extracted by SFMAE was calculated to be 12.5 g. On the other hand, the amount of CO_2 liberated in the atmosphere for each mL of oil extracted through HD was calculated to be 720 g (equivalent to the CO_2 generated by a passenger car driven for 5.5 kms). In other words each mL of oil extracted using HD is equivalent to the CO_2 generated by a passenger car if driven for 5.5 kms. These calculations have been made according to the literature: to obtain 1 kW h from coal or fuel, i.e. 800 g of CO_2 will be emitted to the atmosphere during combustion of fossil fuels (Kala et al., 2017). The cost of running the machinery in terms of energy consumption was calculated by considering the non-domestic electricity tariff for the FY 2018–19 as released by Chhattisgarh State Power Distribution Company Limited. The electricity cost of extraction of oil by SFMAE shall be Rs 0.1 per mL whereas for HD method the cost shall shoot upto Rs 5 per mL of oil. The above electricity cost is tentative and is likely to vary in

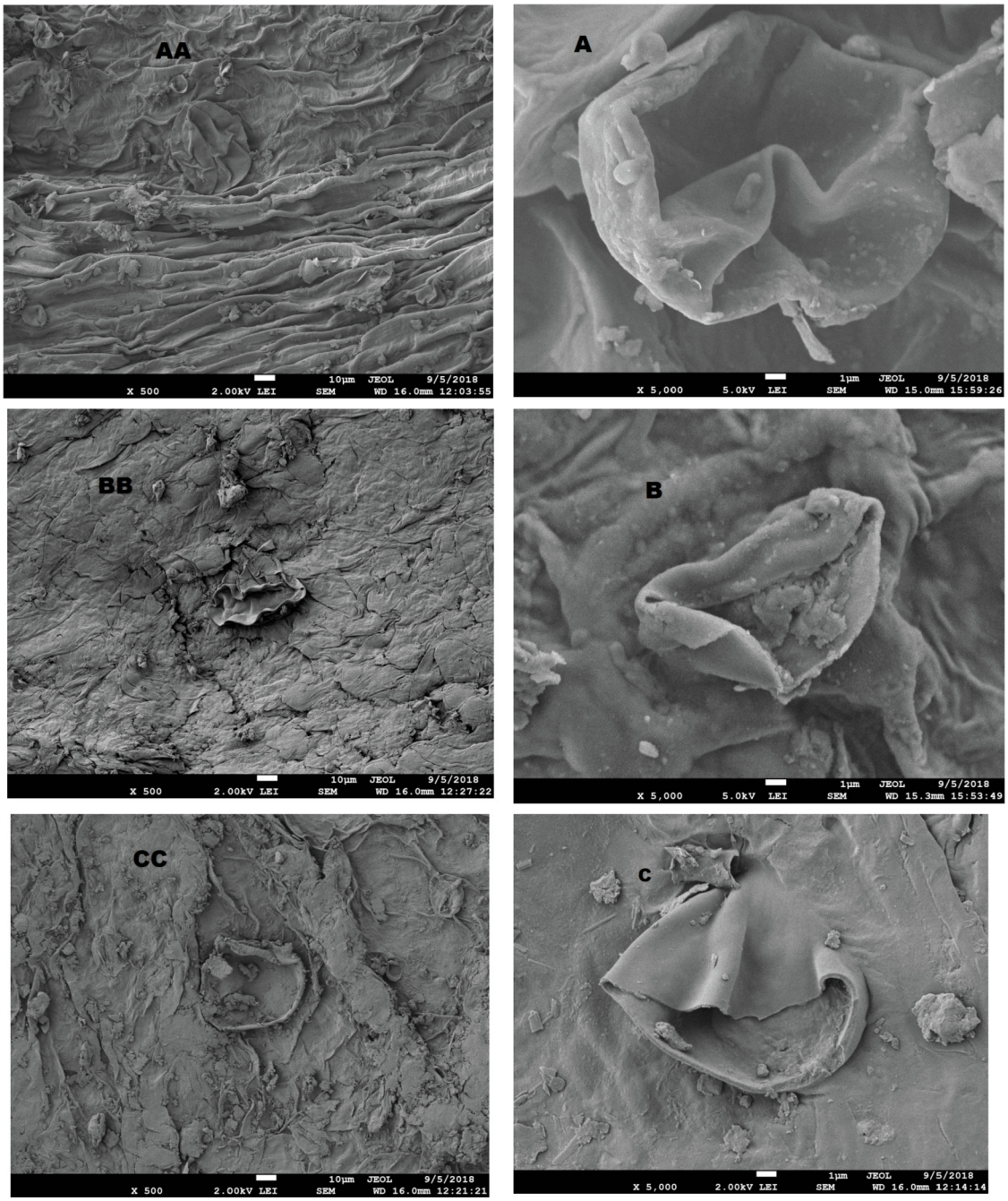

Fig. 7. Scanning electron micrographs revealing microstructural changes during extraction. AA: represents morphology of oil gland at 500 magnification, control sample (dried untreated mentha leaves). A: represents morphology of oil gland at 5000 magnification, control sample (dried untreated mentha leaves). Oil glands appear healthy. BB: represents morphology of oil gland at 500 magnification, dried mentha leaves which was subjected to HD. Oil glands reflects shrinkage. B: represents morphology of oil gland at 5000 magnification, dried mentha leaves which was subjected to HD. Oil glands reflects extreme shrinkage. CC: represents morphology of oil gland at 500 magnification, dried mentha leaves which was subjected to gravity assisted SFMAE. Oil glands appears de-configured. C: represents morphology of oil gland at 5000 magnification, dried mentha leaves which was subjected to gravity assisted SFMAE. Complete rupture of oil gland conclusive.

different states of India as electricity tariff is not uniform for every state and depends upon the power production of that particular state and other policies.

4. Conclusion

The unique concept of microwave surge which is being applied for the first time in extraction of essential oil paid well. An extraction yield of 15.2% w/w was obtained in 6 min of 20% microwave power upon prior impacting the biomass with microwave surge for 4 min (2 min with 60% power followed by 2 min 40% power). Whereas, on the other hand five times lesser yield was obtained from 6 h of conventional HD. The striking feature was both increase in extraction yield and significant reduction in extraction time as well. Noteworthy, to mention that when microwave surge was not implemented, extraction yield was similar to that obtained from HD method. The work also validated that increase in yield of oil was also supported by increased extraction of volatile principles

(both oxygenated and non-oxygenated compounds) which in turn also indicated better biological activity in terms of antioxidant potential when compared to the oil obtained from HD method. Thus the results of extraction yield were found to be in tandem with the oil quality (volatile principles and biological activity). The most striking outcome was ensuring the integrity of the biomass. Using the surge concept, the nutraceutical principles present in the biomass was not compromised during the oil extraction and was thus available for further extraction. Results (Table 4) indicated that the yield of nutraceutical principles from microwave surge treated biomass were comparable to that obtained from control sample (untreated sample). Whereas prolonged heating of the biomass during HD caused a considerable degradation of the said principles thus rendering the biomass unfit for any further use. The success of the proposed technology lies in executing/impacting sudden rupture of oil gland by high microwave irradiation for a shorter period of time followed by sustained gravity assisted drainage of oil under low microwave power without compromising the integrity of the biomass to protect its re-usability opportunities. Quality of oil should be of utmost priority and the process performance should be judged based on the increased yield of oxygenated compounds as they contribute immensely towards building overall oil quality. In light of the above fact, any technological valorization directed towards quick rupture of oil gland in a solvent free environment without compromising biomass integrity shall definitely add value and potential to the proposed technology. Such technology is the need of the 21st century to ensure sustainable development by keeping technology, environment and consumers in the same frame which will foster healthy competition among industries and compel them to be greener and innovative.

Conflicts of interest

The authors declare that there is no conflict of interest associated with this article.

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